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6. AUTHOR(S)
Z. Boulos, M.C. Moore-Ede

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Institute for Circadian Physiology
677 Beacon Street
Boston, MA 0225-3203

8. PERFORMING ORGANIZATION REPORT NUMBER
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13. ABSTRACT *(Maximum 200 words)*

An attempt was made to accelerate the reentrainment of circadian rhythms in squirrel monkeys exposed to 8-hr phase advances and phase delays of the daily light-dark cycle by timed administration of the short-acting benzodiazepine, triazolam. On the day of the phase advance, each animal received a single injection of triazolam (0.3 mg) or of vehicle alone in mid-subjective day, 2 hr after the new time of dark onset, while on the day of the phase delay, the animals received triazolam or vehicle in late subjective night, just before dark onset. The daily acrophases of the circadian rhythm of body temperature were calculated by cosinor analysis, and exponential functions were fitted to the acrophases that followed each of the phase shifts. The rates of reentrainment, defined as the time required for the exponential functions to reach 90% of their asymptotic values, were slower after the phase advance than after the phase delay but did not differ significantly between drug and vehicle conditions. Thus, unlike the case in rodents, triazolam fails to alter circadian reentrainment rates in diurnal primates.

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FINAL TECHNICAL REPORT

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PHARMACOLOGICAL RESETTING OF THE CIRCADIAN SLEEP-WAKE CYCLE
Effects of Triazolam on Reentrainment of Circadian Rhythms in a diurnal primate

Triazolam is one of several benzodiazepines found to be effective in resetting the circadian rhythm of locomotor activity in hamsters [Alprazolam (17); Diazepam (1); Midazolam (16); Triazolam (9)]. Single injections of triazolam cause phase advances when administered during the middle of the hamster's subjective day, and phase delays when administered during subjective night and early subjective day (9). These effects are dose-dependent (10), and are obtained in constant light (LL), constant darkness (DD), and in blinded animals (9,12). Furthermore, administration of a single dose of triazolam in mid-subjective day on the day of an 8-hr phase advance of a daily LD cycle reduces the time required for hamsters to reentrain to the new cycle by about 50% (13). These properties of triazolam and other benzodiazepines, combined with their low toxicity, make them potential candidates for use in the treatment of human circadian disorders, including those associated with jet travel and with changes in shiftwork schedules.

Unlike the case in humans, where triazolam has sedative-hypnotic effects, in hamsters this short-acting benzodiazepine induces hyperactivity, and recent evidence indicates that at least some of the phase shifting effects of triazolam are mediated by its effects on behavioral activity. Thus, immobilizing hamsters immediately after triazolam administration abolishes the phase advancing and phase delaying effects of the drug (14), whereas inducing hamsters to run in activity wheels at different circadian phases causes phase shifts similar to those caused by triazolam (4,7). Inducing locomotor activity in mid-subjective day has also been shown to greatly reduce reentrainment time following an 8-hr advance of the daily LD cycle (5,6).

In a recent study in squirrel monkeys, triazolam administration in LL caused phase-dependent phase shifts similar to those obtained in hamsters (3). Phase advances were obtained after injections in mid- to late subjective day, and phase delays after injections during subjective night and early subjective day. The phase shifts cannot be attributed to an increase in behavioral activity, as triazolam causes sedation in these diurnal primates. The aim of the present study was to determine whether properly timed triazolam injections are also effective in accelerating the reentrainment of circadian rhythms in squirrel monkeys following 8-hr phase advances s

and phase delays of the LD cycle.

METHOD

Six adult male squirrel monkeys (Saimiri sciureus) weighing 725-1040 g at the time of surgery were studied. During experimental sessions, the animals were individually maintained in stainless steel cages (45x45x60 cm), each enclosed in a light-tight, sound-attenuating, ventilated wooden chamber. Lighting in the chambers was provided by a 22-watt circular fluorescent lamp partially covered with electrical tape to reduce illumination intensity to 60 lux.

Body temperature and locomotor activity were recorded at 10 min intervals by telemetry, using battery-operated transmitters (Mini-Mitter model VM-FH disc with extended life batteries) implanted in the peritoneal cavity. Only the temperature data are presented in this report, as the daily locomotor activity patterns were highly variable and subject to strong masking effects by light and darkness.

The monkeys were anesthetized with Halothane and the transmitters were surgically implanted in the peritoneal cavity under sterile conditions. The animals were allowed a minimum of 2 weeks for recovery, during which time they were kept in the colony room under LD 12:12 (L: 0800-2000 hr, EST). They were then transferred to the isolation chambers and kept under the same LD cycle for 10 days. On day 11, the LD cycle was advanced by 8 hr, by shortening the duration of the daily light segment, and each animal received an intraperitoneal injection of 0.3 mg triazolam in 0.5 ml dimethyl sulfoxide (DMSO) vehicle (N=3) or of vehicle alone (N=3). The injections were administered at 1400 hr (+/- 0.5 hr), 2 hr after the new time of dark onset, with the aid of an infrared light source and viewer (FJW Optical Systems). On day 27, the LD cycle was delayed by 8 hr, by lengthening the duration of the light segment, and the animals received a second injection of triazolam or of vehicle alone between 1930 hr and 2000 hr, just before dark onset. The 2 injection times therefore corresponded to CT6 (Circadian Time 6, where CT0 represents the time of light onset and the beginning of subjective day, and CT12 the time of dark onset and the beginning of subjective night) for the phase advance and CT20 for the phase delay. The animals were allowed 15 days to reentrain to the shifted LD cycle and were then returned to the colony room.

Three weeks later, the monkeys were put back in isolation chambers with the aim of repeating the procedure of the first experimental session, giving triazolam to animals that had previously received vehicle alone, and vice-versa. Two days after the first phase shift/injection, however, one of the monkeys was found lying on the floor of the cage. It was treated with antibiotics and dexamethasone but died overnight (an autopsy report concluded that the animal died from acute peritonitis caused by leakage of intestinal contents in the abdomen, due either to

accidental perforation of the ileum during the injection or to local intestinal necrosis caused by the large numbers of microfilaria found in the abdomen). The following day, a malfunction of the light-scheduling equipment caused a disruption of the LD cycle. The remaining 5 animals were therefore returned to the colony room, and the entire experimental session was repeated 3 weeks later. The animal that died was not replaced.

The daily phases of the circadian rhythm of body temperature were obtained by cosinor analysis, which is included in the Dataquest III software. A 24-hr cosine function was fitted to consecutive 24-hr data segments by least-squares, and the time of the peak of the fitted curve used to index the daily acrophase of the rhythm. Reentrainment rates were estimated by fitting exponential functions to the acrophase for the day before the phase shift and to the 8 acrophases that followed the phase shift (the acrophase for the day of the phase shift was omitted, as it would have been influenced by the direct effect of triazolam on body temperature), and calculating the time required for the fitted function to reach 90% of its asymptotic value. Analysis of variance (2-factor repeated measures design) was performed on the acrophase data, with each animal serving as its own control.

RESULTS

All monkeys showed stable entrained temperature rhythms with a daily range of 2-3°C. Body temperature started to increase 1-2 hr before lights-on, reaching maximum levels shortly before the end of the light segment. The daily acrophases obtained by cosinor analysis preceded dark onset by about 5 hr.

The temperature records of a representative animal during drug and vehicle conditions are shown in Figure 1. Triazolam administration had a marked sedative effect which lasted 2-3 hr. During that time, the monkeys were usually found lying on the cage floor, rather than in their typical sleep position (crouching with head tucked between the legs), and they showed little or no response to external stimulation (noise or touch). Sedation was accompanied by a lowering of body temperature, especially during the phase advance portion of the study when the injections were administered in the middle of the high temperature phase of the rhythm.

Light exerted a direct, masking effect on body temperature in all animals. Thus, on the first few days after the LD cycle was advanced, light onset was immediately followed by a moderate increase in temperature, but temperature increased again several hours later, reaching its normal daytime level a little earlier each day. A similar, gradual advance was seen in the timing of the daily fall in temperature. Following the phase delay, temperature started to increase a few hours later on each of the next 3-4 days,

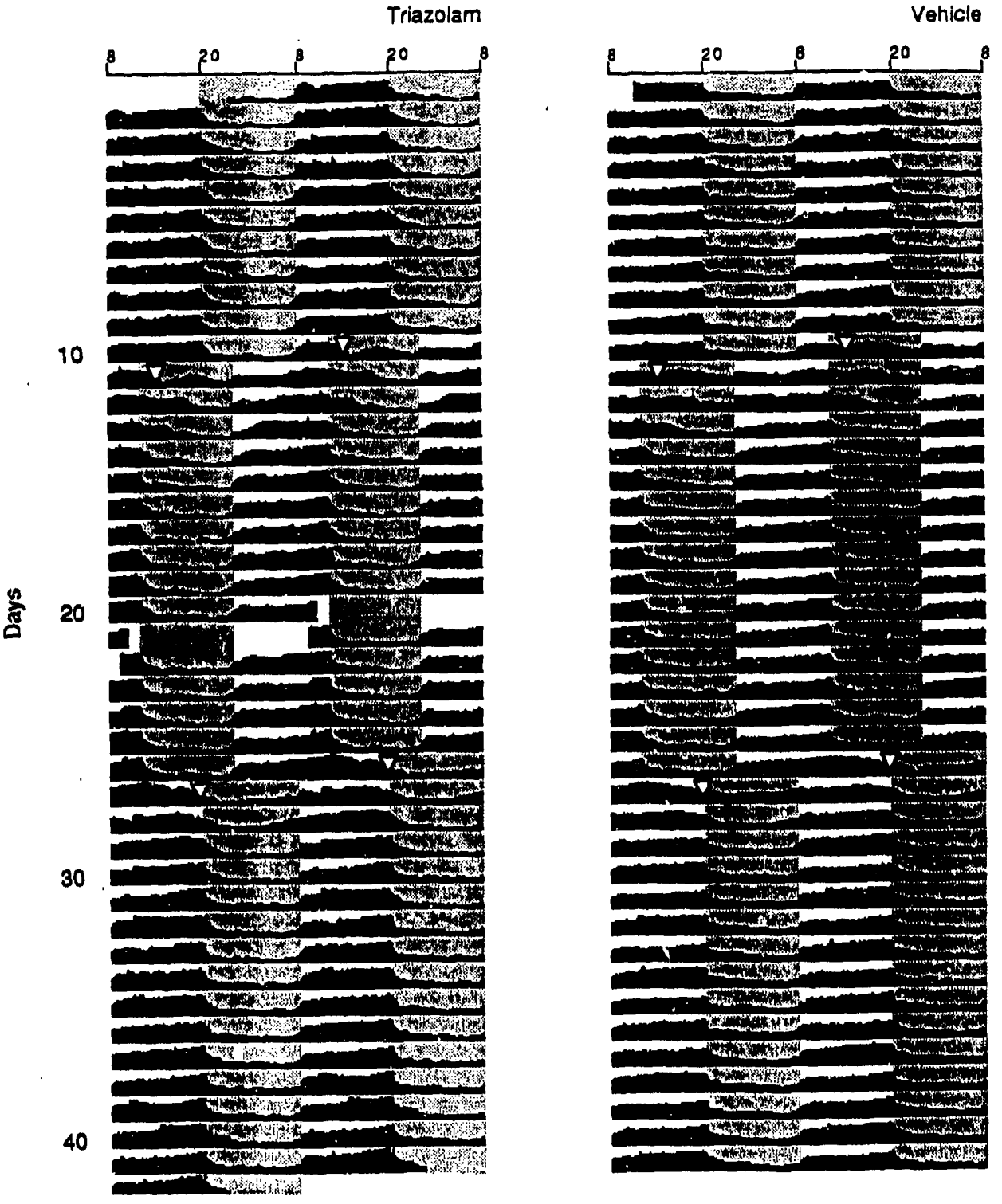

Figure 1. Double-plotted records of body temperature for monkey M981 during triazolam and vehicle conditions. The daily LD cycle (hours of darkness indicated by shading) was advanced by 8 hr on day 11 and delayed by 8 hr on day 27. Injection times are indicated by inverted triangles.

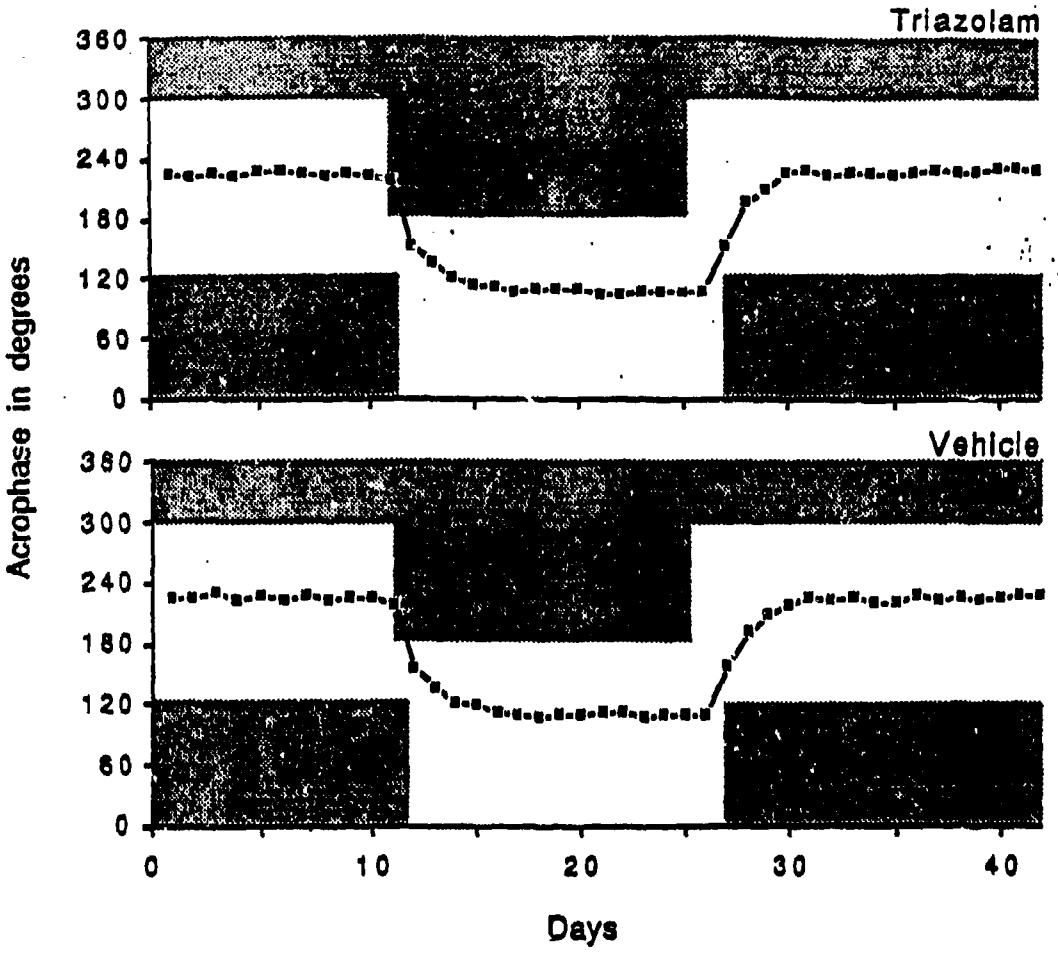

Figure 2. Mean daily acrophases in degrees (0 degrees represents midnight) for the 5 squirrel monkeys during triazolam and vehicle conditions. Shading indicates the dark portion of the LD cycle.

but remained at a high level until dark onset.

Figure 2 shows the mean daily acrophases during drug and vehicle conditions for the 5 monkeys that completed the study. The 2 sets of data are virtually identical. Analysis of variance on the first 8 acrophases following the phase shifts showed a highly significant Time effect ($p<.0001$ for both advances and delays), but no significant Drug effect ($p>.05$) or Drug x Time interaction ($p>.05$) for either advances or delays.

The parameters of the exponential functions fitted to the daily acrophases for each animal and for the entire group under each condition are given in Table 1. Reentrainment rates, defined as the time required for the exponential function to reach 90% of its asymptotic value, varied widely between animals, but within animals the values for drug and vehicle conditions were remarkably similar. For example, during the phase advance portion of the study, monkey M982 was the fastest to reentrain after both triazolam and vehicle injections (0.96 and 0.95 days, respectively), while monkey M990 was the slowest (6.94 and 7.02 days). For the group as a whole, advances required 3.52 days after triazolam and 3.71 days after vehicle administration, while delays required 2.53 days (triazolam) and 2.39 days (vehicle).

Although the direct effect of light did not entirely mask the endogenous oscillation in body temperature, it could have affected the daily acrophase determinations sufficiently so as to obscure any effect of triazolam on reentrainment rate. To examine this possibility, we compared body temperature at the time of light onset following drug and vehicle administration. Since temperature in the entrained steady-state starts to rise before light onset, this measure provides an index of circadian entrainment that is not affected by masking. Figure 3 shows that temperature at lights-on decreased following the phase advance and increased following the phase delay, returning to its pre-shift level approximately 8 and 4 days later, respectively. Even by this criterion, however, reentrainment rates after triazolam and vehicle administration did not differ significantly. Analysis of variance showed a strong Time effect ($p<.0001$), but no significant Drug effect or Drug x Time interaction ($p>.05$) for either advances or delays.

DISCUSSION

The failure of triazolam to accelerate circadian reentrainment was unexpected, as the injection phases (CT6 and CT20) corresponded to those at which injections in LL caused phase shifts of up to 3 hr in magnitude (3). Vehicle-injected animals reentrained at an average rate of 2.2 hr/day during the phase advance and 3.3 hr/day during the phase delay, and a 3-hr triazolam-induced phase shift would therefore have reduced reentrainment time by about a day in both conditions. The triazolam dose used in the present study (0.3 mg) was higher than that used in LL (0.2 mg), but it was chosen only after a pilot experiment failed to show any effect at the lower dose.

Table 1. Parameters of exponential function fitted to daily acrophases.

		TRIAZOLAM				VEHICLE			
	Monkey	A	k	C	t(90%)	A	k	C	t(90%)
ADVANCE	M981	115.95	-0.46	114.26	3.79	126.43	-0.44	112.29	4.33
	M968	116.87	-0.46	92.87	3.16	127.01	-0.48	91.39	3.85
	M990	126.45	-0.25	102.35	6.94	131.90	-0.23	106.16	7.02
	M982	121.20	-1.10	106.66	0.96	119.29	-1.23	106.15	0.95
	M987	115.80	-0.64	106.16	2.55	109.20	-0.83	110.08	1.69
	Mean	117.12	-0.48	105.50	3.52	118.13	-0.49	107.83	3.71
DELAY	M981	-113.98	-1.04	233.24	1.22	-122.05	-0.72	235.47	2.13
	M968	-124.88	-0.56	216.49	3.23	-117.00	-0.53	213.63	3.20
	M990	-121.78	-0.64	227.24	2.59	-114.35	-0.58	228.80	2.39
	M982	-114.13	-0.74	226.75	2.00	-122.18	-0.65	224.58	2.24
	M987	-128.10	-0.72	230.12	2.27	-111.67	-0.84	225.61	1.73
	Mean	-120.45	-0.71	226.72	2.53	-117.12	-0.65	225.48	2.39

The exponential function, Aekt+C, was fitted to the individual and mean daily acrophases that followed an 8-hr phase advance and an 8-hr phase delay of the LD cycle for triazolam and vehicle conditions. In the equation, k is the time constant, t the time in days, and C the asymptotic value of the fitted function in degrees (representing the final steady-state acrophase). Also shown is the time in days required for the fitted function to reach 90% of its asymptotic value (t(90%)).

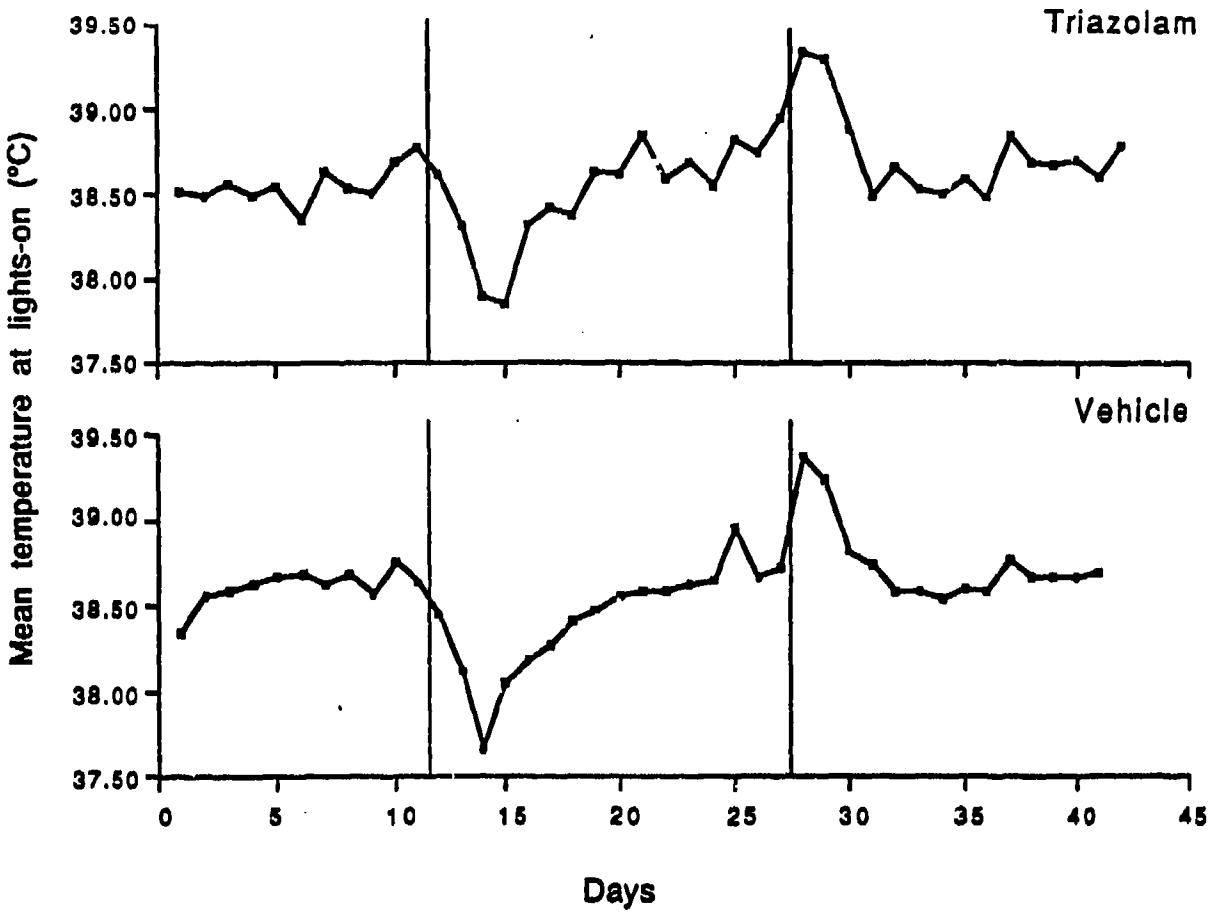

Figure 3. Mean body temperature at lights-on for the 5 squirrel monkeys during triazolam and vehicle conditions. The days of the phase shifts are indicated by vertical lines (day 11: phase advance; day 27: phase delay).

There is, however, another difference between the 2 studies: our injections were administered either in total darkness or just before dark onset, whereas all injections in the earlier study were administered in the light. There is evidence that the circadian effects of some benzodiazepines are strongly light-dependent. The clearest example is diazepam, which causes phase-dependent phase shifts in hamsters maintained in LL (1) but has no effect in blinded hamsters (2). In the case of triazolam, the similarity between the phase shifts obtained in LL, DD, and in blinded hamsters would suggest that these effects are independent of lighting condition. However, this does not preclude the possibility that the circadian effects of triazolam in squirrel monkeys are light-dependent, since triazolam-induced phase shifts in hamsters are largely attributable to the stimulatory effects of this benzodiazepine on behavioral activity (14).

Triazolam has also been given to human subjects in an attempt to facilitate their adjustment to a shift in sleep-wake schedule. In one study (15), rotating shift workers were administered triazolam at bedtime on the first 2 days of their night shift tour. Triazolam increased sleep efficiency and total sleep time on these 2 days, but daytime sleep was not facilitated on the next 2 drug-free days. In another study (8), subjects received triazolam at bedtime on the first 3 days following a 12-hr shift in sleep-wake schedule. Triazolam resulted in a significant improvement in sleep, as well as in alertness and performance during the shifted wake period, but it is not clear whether the latter effect was due to the increase in prior sleep efficiency or to faster reentrainment rates.

More recently, subjects were given triazolam on the 5 days following an 8-hr delay of their sleep-wake and light-dark schedule (11). On the first day, triazolam was given 3 hr before the new bed- and dark onset time, and resulted in a delay in the end of the quiescent period for plasma cortisol and in the daily rise of plasma melatonin. On the next 4 days, triazolam was given at bedtime, but the differences between triazolam and placebo conditions on the third day were no longer significant, despite the fact that neither hormonal rhythm had fully reentrained by that time. Thus, the delay achieved on day 1 does not appear to have been a permanent or sustained phase shift.

In summary, the available data indicate that triazolam administration may facilitate the adaptation to abrupt shifts in sleep-wake or light-dark schedules in humans by increasing sleep efficiency, but there little or no evidence in either human (8,11,15) or non-human primates that triazolam accelerates the reentrainment of circadian rhythms.

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Personnel Associated with this Research

M.C. Moore-Ede, M.D., Ph.D., Principal Investigator
Z. Boulos, Ph.D., Co-Principal Investigator
T.A. Houpt, Graduate Student
M.-E. Natale, Research Technician

Publications

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